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Larvae of the western budworm, *Choristoneura occidentalis*, displayed symptoms of hyperactivity, regurgitation, and reflex bleeding after topical application of 0.4 μg per 100 mg body weight of Lannate® (*S*-methyl *N*-[(methylcarbamoyl) oxy] thioacetimidate). When the larvae were kept in containers with non-absorbent surfaces, the ejected hemolymph and regurgitated fluids enveloped the larvae and mortality increased. The greater time required for the drying or clotting of ejected fluids in closed containers appeared related to the higher mortality which also occurred in closed containers. Fluorescent tracer methods were used to show that ejected fluids did not readily penetrate the tracheal system. However, when spiracles and cuticles of larvae were covered by the ejected fluids, both CO_2 production and O_2 consumption rates decreased below normal during prostration. When prostrate larvae were on an absorbent surface, respiration rates were well above normal.

The first visual symptoms of Lannate poisoning in topically-treated western budworm larvae (*Choristoneura occidentalis* Freeman) are hyperactivity, regurgitation, and bleeding. The hyperactive state is followed by prostration and eventual recovery or death. This behavior is also characteristic of healthy larvae subjected to a physical stimulus, such as crowding. If the disturbance is momentary, the budworm larvae will re-ingest the regurgitated fluid. But if the disturbance is continued, the prolonged hyperactivity often leads to a rupture of the integument of the prothorax from which hemolymph is expelled. This may be a form of reflex bleeding, a defense mechanism of some insects described by Wigglesworth (1965).

The regurgitation and reflex bleeding responses of western budworm larvae to insecticide poisoning are not atypical. Beard (1958) observed regurgitation after larvae of *Galleria mellonella* (L.) were injected with DDT. He found that regurgitation was caused by DDT-induced spasms of the gut and that larvae that were covered with regurgitated fluids became rigidly prostrate and died if not washed with water shortly thereafter. Chadbourne and Rainwater (1953) reported that *Heliothis armigera* (Hbn.) became wet with liquid from the mouth, anus, and integument when treated with DDT and dieldrin.

Jochum (1956) suggested that the high loss of water resulting from reflex spitting and bleeding of organophosphate-poisoned *Bombyx* larvae was a means of in-

creasing metabolism. This condition enabled the insect to move rapidly and act defensively.

The DuPont carbamate Lannate® (*S*-methyl *N*-[(methylcarbamoyl) oxy] thioacetimidate) can produce the intoxication symptoms in 95% of the treated budworm larvae at a dosage of 0.6 μg per 100 mg body weight. Only 40% of these larvae die, the survivors usually recovering within 24 hours.

However, the mortality rate could be increased to 88% by removing the filter paper lining of the test containers. This suggested that in the absence of absorbing paper, the ejected fluids that bathed the poisoned larvae inhibited the exchange of gases and hence aided toxicity.

The purpose of this study was to determine whether ejected fluids increased budworm mortality by (1) penetrating the tracheal system, (2) covering the spiracles and cuticle to produce a suffocating effect, or (3) spreading insecticide to more sensitive areas of the body. Possible chemical alterations of the insecticide in the presence of hemolymph or regurgitated fluid were not studied.

METHODS AND MATERIALS

Sixth instar western budworm larvae were used in all experiments. The insects were reared in the laboratory in plastic petri dishes and fed a synthetic diet according to the procedures of Lyon and Flake (1965). All treatments were conducted in 100 × 200 mm plastic petri dishes with or without 9.0 cm Whatman No. 1 filter papers lining the bottoms of the dishes. Paper-lined dishes were considered absorbent; unlined dishes were considered non-absorbent.

Topical application of Lannate in acetone was made with a microburet syringe 5.82 manufactured by Micro-Metric Instrument Company. A dosage of 0.4 μg per 100 mg body weight was applied to the dorsal thorax of the larvae. The larvae, weighed to the nearest milligram, had a mean weight of 150 mg. Total larval mortality was recorded 7 days after insecticide treatment.

To determine the volume of hemolymph and regurgitated fluids ejected by intoxicated larvae, 26 larvae were weighed, placed in separate containers, and treated with Lannate. An hour after treatment the insects were again weighed and the resulting fluid loss determined in milligrams.

To compare the clotting times of budworm hemolymph in closed and open containers, 25 μl hemolymph samples were drawn from healthy larvae and spread over a 283 mm^2 area (the area of a penny) in plastic petri dishes with and without covers. The spots of hemolymph were periodically touched with a dissecting needle to determine if they had dried.

Tracheal penetration in treated larvae was observed with the aid of the water-soluble fluorescent dye pyrene trisodium sulfonate (3-hydroxy-5,8,10-trisodium sulfonate), which dissolved readily in ejected fluids. Twenty-six larvae were treated with Lannate and placed in non-absorbent dishes that had been dusted with the fluorescent dye. The larvae were dissected under ultra-violet light 1 to 2 hours after treatment and their tracheae were observed.

The oxygen uptake of Lannate-treated larvae was determined with a Gilson respirometer. The treated larvae were placed in one-armed reaction vessels in a water bath maintained at 25° C. A filter paper wick placed in the center well and soaked in 0.20 ml of 10% KOH served as CO_2 absorbent. Respirometer readings were taken at 5 min intervals for 2 hours after treatment

Carbon dioxide production of Lannate-treated larvae was determined with a Beckman Infrared CO_2 Analyzer. The oxygen supply for the system was passed through two CO_2 traps. The first trap contained water acidified with HCl, which humidified the insect holding chamber. The second trap contained 10% potassium hydroxide, which removed remaining CO_2 from the oxygen before it passed through the insect holding chamber. The holding chamber consisted of a length of tygon tubing plugged with glass wool at both ends. A strip of filter paper was inserted into the holding chamber to absorb insect fluids. Before entering the CO_2 analyzer, the air flowing from the insect chamber was dehumidified by passing it through a column of magnesium perchlorate and Drierite® (80—100 mesh). A baseline was established by passing pure oxygen through the system for 10—15 min before the larvae were introduced. The larvae were placed in the holding chamber and their normal CO_2 production measured for 30 min. Then the larvae were treated topically with Lannate by introducing a hypodermic needle through the tygon tubing. The transparency of the tubing permitted visual observations to be correlated with graphic recordings. Observations were made from time of treatment until 60 min after larvae became prostrate. The carbon dioxide production rates of larvae bathed with injected fluids and larvae held on absorbent paper were measured.

RESULTS

The mean weight loss of hemolymph and regurgitated fluids ejected by Lannate-treated larvae was 52 mg or a fluid loss of approximately 52 µl. The mean fluid loss amounted to 35% of the mean larval weight of 143 mg. Twenty-one of the 24 larvae ejected both hemolymph and gut fluid.

Both hemolymph (pH 7) and regurgitated fluid (pH 9—10) are able to wet the lipophilic cuticle of the larvae. In the absence of an insecticide, ejected fluids alone produced mortality among larvae crowded into closed, non-absorbing containers. In one case, the hyperactivity and ejection of body fluids by 63 larvae crowded into a small container caused 87% mortality. In a similar case, 38% mortality occurred among 36 larvae. The effect that an absorbent substrate has on mortality is shown in larval mortality counts made on the seventh day after Lannate application (Table 1). In covered, non-absorbent containers where minimal evaporation occurred, mortality was highest. In open, non-absorbent containers where increased evaporation occurred, mortality was moderate. Mortality was lowest in covered containers in which all ejected fluids were absorbed by filter paper.

The clotting time of budworm hemolymph appeared to be affected by exposure to air. Fifteen samples of hemolymph in open dishes dried three times faster than

TABLE I

Mortality as a function of absorbent surface and evaporation

Treatment	Container Type	7th Day Total Mortality		
		Insects Treated (No.)	Insects Dead (No.)	Mortality (%)
None	Control: Non-Absorbent, Closed Dish	75	7	9
Lannate*	Absorbent, Closed Dish	50	12	24
"	Non-Absorbent, Closed Dish	50	40	80
"	Non-Absorbent, Open Dish	50	22	44

* 0.4 μg/100 mg body wt.

an equal number of samples in closed dishes. Hemolymph in closed dishes required an average of 122 min to dry, while hemolymph in open dishes dried in an average of 46 min.

Regurgitated fluid and hemolymph penetrated only slightly into the tracheal system of budworm larvae. Sixteen larvae showed no penetration and nine showed fluorescent fluid in one or two trachea. Before dissecting the larvae, it was observed under the ultraviolet light that 21 of the 25 Lannate-treated larvae had 14—18 spiracles covered with ejected fluid and three larvae had 8—11 spiracles covered.

Apparently, the wet, ejected fluids can block the spiracular opening without actually penetrating the tracheal system. Fifteen larvae, prostrate because of crowding, regained normal activity after ejected fluids had been rinsed from their bodies. When 20 budworm larvae were momentarily anesthetized with CO_2 and all their spiracles were covered with drops of Elmer's glue, all of the larvae were inactive until the glue dried. However, the hardening glue tended to separate from the cuticle, and 16 of the larvae regained normal activity. The glue adhered well to four of the larvae, and these insects subsequently became moribund. It was observed that hemolymph behaved in a similar manner. As it clotted or dried on prostrate larvae, it no longer adhered to the body but formed a thin lacquer-like film which cracked and separated from the cuticle.

The effect of ejected fluids on oxygen uptake of Lannate-treated larvae is shown in Fig. 1. Three flasks were used per test. One flask held an untreated larva on a glass surface. The second held a treated larva on a glass surface, and the third held a treated larva on a surface covered with shredded absorbent paper. From 10 to 15 min lapsed between treatment and placement of insects in the respirometer. This lapse was followed by a 10-min interval of temperature and pressure adjustment. Consequently, all respirometer readings began 30 min after treatment. Light re-

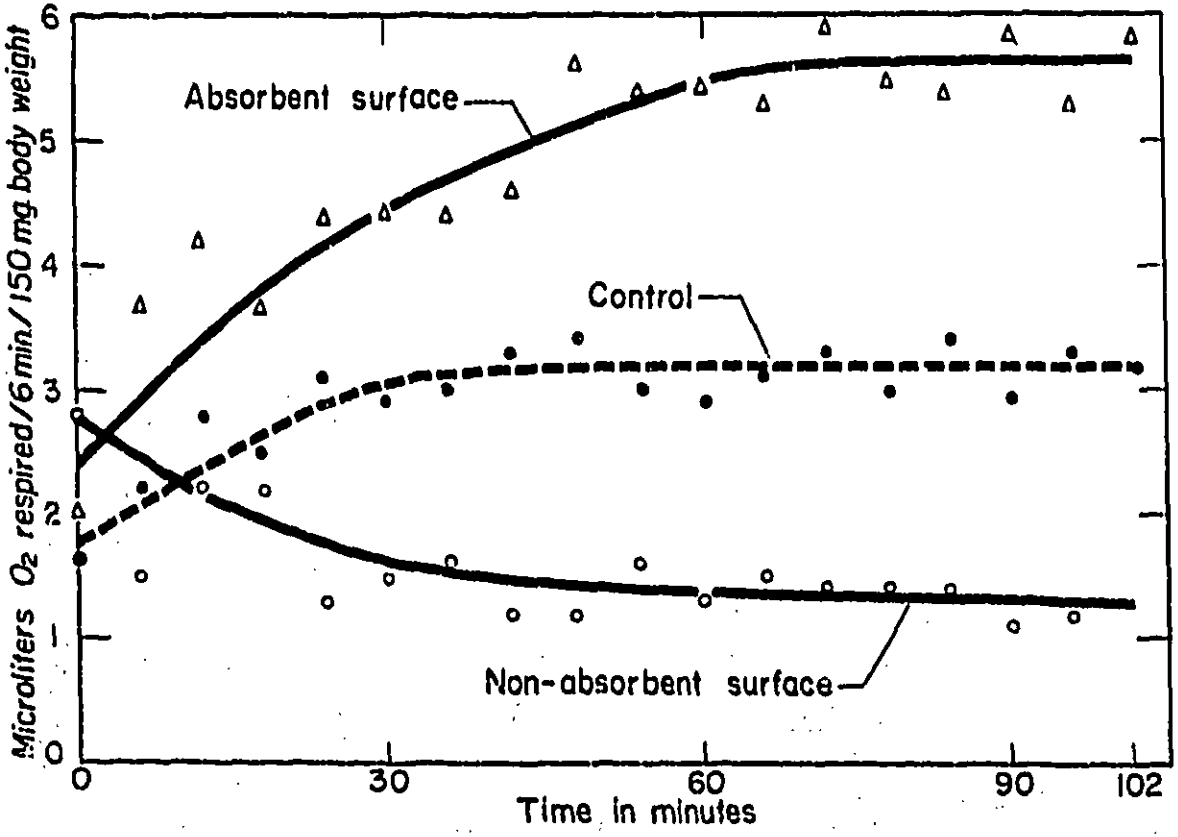

Fig. 1.—O_2 respired by control and Lannate-poisoned larvae on absorbent and non-absorbent surfaces.

fraction by the water bath made it difficult to correlate respiration recordings with insect activity. However, 20 observations outside the respirometer showed that the larvae become prostrate, on an average, 33 min after treatment. Therefore, these respiratory data were obtained during prostration.

In the first 45 min of prostration, the larvae treated on absorbent surfaces showed an increasing respiration rate. Thereafter, oxygen consumption continued at a fairly constant rate that was about 74% higher than the respiration rate of control larvae. The respiration rate of larvae treated on non-absorbent surfaces and bathed in ejected fluids showed a considerable steady decrease during the first 30 min of prostration. However during the following 60 min, respiration dropped more gradually and oxygen consumption was approximately 58% below that of control larvae.

Fifteen larvae to be observed for CO_2 production were kept in the test chambers for 30 min before treatment to establish control respiration rates. Fig. 2 shows the average CO_2 production of these 15 larvae from time of treatment until 60 min

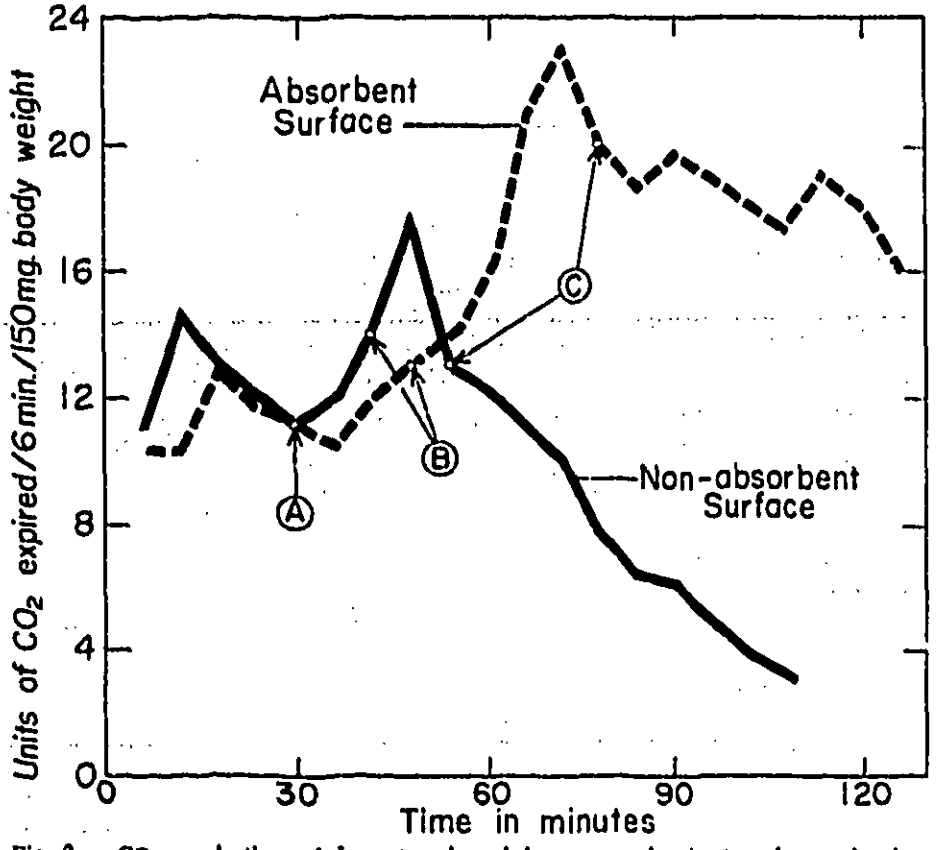

Fig. 2. —CO_2 production of Lannate-poisoned larvae on absorbent and non-absorbent surfaces. (A) Time Lannate applied, (B) Beginning of hyperactivity, (C) Onset of prostration.

after prostration. The larvae that were on a non-absorbent surface became hyperactive an average of 12 min after treatment and remained hyperactive for an average of 12 min. The maximum CO_2 production, approximately 56% above control, occurred during the hyperactive stage. Prostration began an average of 6 min after maximum CO_2 production. After an hour of prostration, the continuously decreasing CO_2 production was 74% below the control rate. Hyperactivity in larvae maintained on an absorbent surface began an average of 18 min after treatment and continued for approximately 30 min. The maximum CO_2 production was 85% above the control rate. Prostration occurred within an average of 8 min after maximum CO_2 production. Though it gradually decreased, the CO_2 production rate during prostration remained well above the control rate.

The absorbent paper was removed from the holding chambers after 3 Lannate-treated larvae had been prostrate for 18 min. While on the absorbent paper, their average rate of CO_2 production during prostration was 68% above the control rate. After the absorbent paper was removed, regurgitated fluid and hemolymph drawn from healthy larvae were injected into the holding chambers. Within 30 min, the CO_2 production rate of the prostrate larvae had decreased 47% below the control rate.

Dissections of prostrate larvae bathed in ejected fluids containing fluorescent dye showed that 80% of these larvae re-ingested some of the ejected body fluids. Because Lannate is water-soluble, it was possible that the Lannate applied to the cuticle dissolved in ejected body fluids and was ingested. To determine if ejected fluids were enabling topically applied Lannate to reach the stomach, 60 larvae were rinsed with water 10 min after being topically treated with Lannate. The purpose of the rinse was to remove excess Lannate from the cuticle. Half of the treated larvae were placed in absorbent dishes and the other half were placed in non-absorbent dishes. As expected, mortality among the rinsed larvae dropped. However, their mortality on non-absorbent surfaces was still 36% higher than that of larvae held on an absorbent surface (Table II). Although stomach toxicity may be a factor in increased mortality, the higher mortality of the larvae on absorbent paper is more likely to be due to the suffocating effect of the fluids.

To determine whether hemolymph and regurgitated fluids enhanced toxicity by spreading Lannate to susceptible sites, 50 larvae were treated with Lannate in small

TABLE II

7-day cumulative mortality of Lannate-treated larvae rinsed and not rinsed with water 10 min after treatment

Dosage of Lannate	% Mortality	
	With paper	Without paper
0.4 μg/100 mg body weight — Not rinsed	24	88
0.4 μg/100 mg body weight — Rinsed	10	46

drops distributed over the body. Mortality among this group was 18% by the 7th day. Mortality was 24% in a group of 50 larvae treated on their thoraces with one large drop.

DISCUSSION

Penetration of ejected fluids into the tracheal system did not appear to be the cause of increased mortality among larvae on non-absorbent surfaces. Studies with fluorescent dye showed that body fluids seldom penetrated beyond the spiracles and into the tracheal system even though fluids covered the spiracular openings and the cuticle. Application of Lannate in numerous small drops suggested that ejected fluids did not enhance larval mortality by bringing Lannate into contact with more "sensitive" areas of the body. Nor did incidental ingestion of Lannate dissolved in ejected fluids appear to be a major factor in increased mortality. Moreover, mortality was observed in the absence of any insecticide when crowded, hyperactive larvae became enveloped in their own ejected body fluids.

However, a relationship between mortality and the drying time of ejected fluids was suggested by the decreased mortality of larvae treated in open dishes and by the observation that hemolymph dried three times faster in an open dish than in a closed dish. Mortality decreased when hemolymph, like white glue, dried and pulled away from the cuticle.

Lannate induced in western budworm larvae high respiration rates which reached their peaks during hyperactivity. Though Ingram (1955) found no respiration increase in house flies knocked down by pyrethrins, Lord (1949) found increased oxygen uptake in *Oryzaephilus surinamensis* (L.) poisoned with DDT. Jochum (1956) showed that increased respiration in DDT-poisoned adult *Leptinotarsa decemlineata* Say accompanied the hyperactive stage. Jochum suggested that the increased metabolism associated with high respiration rate was a means of compensating for water loss by producing water of metabolism. Gostick (1961) reported that oxygen uptake increased in adult *Alphitobius laevigatus* F. when treated with malathion, DDT, dieldrin, γ-BHC, allethrin, and DNBP and reached its peak at or before the time of knock-down. Peak respiration in western budworm larvae was reached before the onset of prostration. Prostrate larvae kept on absorbent surfaces exhibited high but very gradually decreasing respiration rates. Larvae kept on non-absorbent surfaces showed an immediate and continuous decrease in respiration after they had become prostrate.

Ejected fluids apparently formed an envelope around the larvae and physically blocked spiracular and cuticular respiration. By inhibiting the higher rates of respiration that normally occur during prostration, ejected fluids appeared to enhance the toxicity of Lannate. Respiration experiments were not carried out on over-crowded larvae. It is possible, however, that the stress of crowding could produce a high respiration rate which might be severely inhibited by the presence of ejected fluids. Such inhibition could be a cause of death among crowded larvae.

ZUSAMMENFASSUNG

WIRKUNG VON ERBRECHEN UND REFLEXBLUTEN AUF DIE STERBLICHKEIT DES WESTLICHEN KNOSPENWICKLERS (CHORISTONEURA OCCIDENTALIS) NACH BEHANDLUNG MIT LANNATE

Die Raupen des Westlichen Knospenwicklers, *Choristoneura occidentalis* Freeman, zeigten nach örtlicher Behandlung mit 0,4 µg pro 100 mg Körpergewicht Lannate® (S-methyl-N-[(methylcarbamoyl) oxy] thioacetimidat) Symptome von Übererregung, Erbrechen und Reflexbluten. Wurden die Larven in Behältern mit nichtabsorbierenden Oberflächen gehalten, so bedeckten die ausgetretene Hämolymphe und die erbrochenen Flüssigkeiten die Larven und erhöhen die Sterblichkeit. Die längere Zeit, die zum Trocknen oder Gerinnen der abgegebenen Flüssigkeiten in geschlossenen Behältern erforderlich war, schien in Beziehung zu der höheren Sterblichkeit zu stehen, die zugleich in geschlossenen Behältern auftrat. Fluoreszenz-Nachweis-Methoden wurden benutzt, um zu zeigen, daß die ausgeschiedenen Flüssigkeiten nicht leicht in das Tracheensystem eindringen. Wenn jedoch die Stigmen und die Kutikula der Raupen mit den abgesonderten Flüssigkeiten verschmiert waren, sanken sowohl die CO_2-Produktion wie die Sauerstoffverbrauchsraten unter das während der Behandlung normale Niveau. Wenn behandelte Larven auf einer absorbierenden Unterlage gehalten wurden, waren die Respirationsraten gut übernormal.

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